U.S. Environmental Protection Agency
Office of Research and Development

National Exposure Research Laboratory
Research Triangle Park, North Carolina
Athens, Georgia
Cincinnati, Ohio
Las Vegas, Nevada

STANDARD OPERATING PROCEDURE (SOP)

Title: Analytical method for non-targeted and suspect screening in environmental and biological samples using Time of Flight Mass Spectrometry (TOFMS)

SOP Number: D-EMMD-PHCB-034-SOP-01	**Effective Date: April 6, 2016**

SOP was Developed: ☒ **In-House** ☐ **Extramurally**

Alternative Identification:

SOP Steward

Name: Mark Strynar

Signature / Date:

Approval

Name: Myriam Medina-Vera

Title: Branch Chief

Signature / Date:

Concurrence*

Name: Sania Tong-Argao

Title: Quality Assurance Manager (EMMD)

Signature / Date:

* To be used by Project Officers for review by the QAM or for Category 'A' projects.

Analytical method for non-targeted and suspect screening in environmental and biological samples using Time of Flight Mass Spectrometry (TOFMS)

Table of Contents

Analytical method for non-targeted and suspect screening in environmental and biological samples using Time of Flight Mass Spectrometry (TOFMS)

1.0 Scope and Application

This standard operating procedure (SOP) describes a method for the non-targeted analysis (NTA) and suspect screening of chemicals in environmental and biological media using Time of Flight Mass Spectrometry (TOFMS). This method will be used to discover xenobiotic chemicals in various environmental and biological media screening against various in-house and OEM purchased data bases. There is much interest in the determination of the chemicals to get a more holistic idea of what we are exposed to in various media that are pertinent to human exposure studies (water, house-dust, soil, serum etc..). Classically this has been done through target analysis of a small number of chemicals of interest in select media. However, newer high-resolution mass spectrometry (HR-MS) techniques (such as TOFMS) allow for the rapid screening of analytes of interest based accurate mass measurements and spectral isotope matching. As the technique is kept consistent through the screening a various media, the result will be the ability to detect chemicals in different media, as well as co-occurrence of chemicals in specific media. This data will help to prioritize existing high-throughput toxicology assays (via NCCT) and modeling efforts (NERL) for chemicals of interest.

2.0 Summary of Method

This method describes the LC/TOFMS analysis for the NTA and suspect screening of chemicals in various environmental and biological media. Refer to previously written SOPs for sample extraction and cleanup techniques. The following SOPs will be followed with minor alterations for NTA and suspect screening efforts. The SOPs include ([1]EMAB-112.0- Extraction and Analysis of Perfluorinated Compounds (PFCs) from Surface Waters by High Performance Liquid Chromatography (HPLC)-Tandem Mass Spectrometry (MS/MS); [2]MDAB-23.0- Standard Operating Procedure (SOP) For Extraction And High Performance Liquid Chromatography/Mass Spectrometry Analysis Of Perfluorinated Acids And Sulfonates From House Dust; and [3]MDAB 077.0- Extraction of Perfluorinated Compounds from Fish Liver Homogenate) for extraction and cleanup techniques. Additional information about the analytical equipment and software details can be found in the manuals (see References).

A portion of the sample extract is added to an auto-sampler vial along with 2 mM ammonium acetate buffer to match starting conditions (75% water: 25% methanol) of the HPLC gradient and is ready for LC/TOFMS analysis.

3.0 Definitions, Acronyms, and Abbreviations

- **Extract**: The sample extract that contains native target analytes and internal Standards.
- **Internal Standard (IS):** The fixed amount of compound that is added to each sample prior to its extraction. If quantitation is to be done the ratio of the detector signal of the native analyte to the detector signal of the IS is compared to ratios obtained from calibration curves where the IS level remains fixed and the native analyte levels vary. The IS is used to correct for minor sample-to-sample differences in extraction, matrix, purification, injection volume, chromatographic behavior, and MS ionization efficiency. All internal standards used in this procedure are mass-labeled, meaning that one or more atoms in the internal standard are replaced with a lesser abundant, higher molecular weight isotope, resulting in a mass difference between the analyte and mass labeled internal standard that allows the mass spectrometer to distinguish between the analyte and internal standard. In particular with NTA and suspect screening analysis, IS chemicals may be used to adjust for chromatographic drift from sample to sample, and to act as additional compounds to judge the mass accuracy of the instrumentations performance.
- **Double Blank:** A blank sample consisting of 100 µL of MeOH and 300 µL of 2 mM ammonium acetate buffer. Double blanks do not undergo complicated sample preparation. Adding the MeOH and buffer to an auto-sampler vial is the only sample preparation required. No internal standard or analytes are added to double blanks. This sample is used to assess solvent, and HPLC vial and cap contribution to analytical contamination.
- **Method Blank:** A blank sample that has undergone all sample preparation (i.e. addition of internal standard, alkaline digestion, and solid phase extraction (SPE)) as water, house-dust, soil or serum samples, but which began with DI water rather than an actual sample.
- **Matrix Blank:** A blank sample that has undergone all sample preparation (i.e. addition of internal standard, alkaline digestion, and SPE) as water, house-dust, soil or serum samples, if a blank matrix is available that is analyte free. In the event no blank matrix that is deemed acceptable, none will be used.
- **Quality Control (QC) Sample:** Depending on the media of choice this may include a standard reference material (SRM) from the National Institute of Standards and Technology (NIST) (house-dust, serum), or a prepared sample with a secondary standard source if available. QC samples undergo the same sample preparation (i.e. addition of internal standard, alkaline digestion, and SPE) as unknown samples. Measurements of quality control samples with each batch of samples gives the analyst a measure of whether or not sample preparation, extraction, and analysis has been performed properly.
- **Calibration standards:** In general calibration standards are not run in NTA and suspect screening efforts with respect to quantitation of target compounds in

environmental and biological media. However, with NTA and suspect screening efforts a series of calibrations compounds are used to mass calibrate the instrument daily before use and to auto-tune the TOFMS instrument. These consist of a series of known compounds in an OEM solution used to assure the mass accuracy of the instrument on a regular basis. In addition, a second set of known compounds (called reference compounds) are continually infused into the TOFMS for real-time mass correction. These are the compounds: purine [exact mass = 120.043596], HP0921 hexakis (1H,1H,3H-tetrafluoropropoxy) phosphazene [exact mass = 921.002522] and tetrahydroperfluorononanoic acid (THPNA) [exact mass = 391.0009]. Depending on the polarity of the instrument and the mobile phase modifiers used, different reference masses are seen. In addition, any known compound with an exact mass may be added that is not expected to be present in the samples being analyzed. Refer to Table 1 for additional references masses and forms used in this analysis.

Table 1: Reference masses for real-time mass correction in TOFMS analysis

Species	Positive Ion m/z	Negative Ion m/z
CF_3 (trifluoro acetic acid TFA) fragment)		68.995758
TFA anion		112.985587
purine	121.050873	119.036320
HP0921	922.009798	
HP0921 (formate adduct)		966.000725
HP0921 (acetate adduct)		980.016375
HP0921 (TFA adduct)		1033.988109
THPFNA		391.0009

- **Unknown Sample:** Each unknown sample is extracted according to the aforementioned SOPs and analyzed along with QA/QC, blank and replicate sample. This may consist of any environmental or biological media (i.e. house-dust, soil, water, serum…) and is compared to other samples with unknown chemical compositions. When available metadata (age, gender, location, upstream/downstream…) will be used to draw conclusions from comparisons to other unknown samples.
- **DI:** Deionized
- **SPE:** Solid phase extraction
- **LTL:** Laboratory team leader

- **LRB:** Laboratory record book
- **PPE:** Personal protective equipment

4.0 Health and Safety

Proper personal protective equipment (PPE) should be used when performing this procedure. PPE that should be worn throughout this procedure include nitrile gloves, safety glasses, and a lab coat. Solvents should be opened and handled in a properly functioning fume hood.

5.0 Cautions

5.1 Contamination of Samples

Care must be taken to avoid sample contamination. Throughout sample preparation, laboratory personnel must remove gloves that have been or are suspected to be contaminated and put on a clean pair of gloves. A clean pipette tip must be used for each sample. Solvents and DI water should be tested to regularly to ensure they are not significant sources of contamination. All glassware and surfaces used in sample preparation must be as clean as possible before usage. Blanks (solvent and method) must be run with each batch of samples to determine what contamination has occurred during sample preparation and analysis. Standards and samples must be capped when not in use to avoid evaporative loss of solvent (MeOH), which could lead to increased concentration of analytes. Standards and samples should not be stored in the same refrigerator or freezer.

5.2 Sample Loss

Loss of some analytes, especially lower carbon chain compounds may occur at high temperatures (greater than 60ºC) and when samples are evaporated to dryness. Therefore, all sample preparation and analysis should occur at or below 60ºC and samples should not be evaporated to dryness (Section 9.6 below). Another potential loss of chemicals is due to sorption to labware and precipitation due to cooling of samples in a refrigerator (4°C). As this in NTA and suspect screening, all unknown compounds cannot be assessed for losses. However, general laboratory practice based on past experience should be taken to help minimize losses. Assessment of losses of already known compounds in samples may be useful to help judge method performance. In addition the use of ISs (see section 3.0) may be beneficial in assessing losses as well.

6.0 Interferences

As HRMS techniques are monitoring for all m/z at the entire time of an analytical

run interferences are expected. This is why it is critical to run solvent and method blanks along with unknown and QA/QC samples. The presence of interferences in particular samples should be assessed by comparisons to blanks. This may be done in several ways. In particular the chemicals found in blank samples may be placed in an "exclusion list" to negate being found in unknown samples and occupy valuable analytical time. In addition, chemicals found in composite of solvent and process blanks can be combined into a personal compound database list (PCDL) with m/z to be sure to eliminate these chemicals based on both m/z and RT rather than simply m/z. This is important as a simple m/z exclusion list may exclude an important mass if it is similar to a chemical found in the blanks. An additional approach is to not exclude any chemicals and when done compare blank samples to unknown samples. Any peak in a sample found to have significantly more signal than in the blank (i.e. S/N > 3:1) could be deemed a real chemical.

7.0 Personnel Qualifications/Responsibilities

Note that this SOP assumes a thorough working knowledge of basic laboratory skills, reagents, and instrumentation. This document is designed to guide a trained laboratory worker in the methodology discussed below and it is not intended to instruct individuals on the basic aspects of analytical chemistry. Performance of procedures described in this SOP requires previous laboratory experience and training. At least 2 years of previous laboratory experience is necessary to perform the procedures described in this SOP and a technical or 4-year degree in chemistry would be desirable. The laboratory worker who performs the sample extractions will be responsible for entering experimental information in the laboratory record books. The Laboratory Team Leader (LTL) will also oversee the sample preparation, extraction, and cleanup, to ensure all SOPs are followed by all project staff. However, this SOP only covers NTA and suspect screening analysis of environmental and biological samples.

8.0 Equipment and Supplies

8.1 Laboratory Equipment

- Fume hood
- Solvent cabinet
- Vortexer
- Nitrogen Generator or clean compressed Nitrogen gas supply (>99% purity)
- Agilent 1100 high pressure liquid chromatography system with autosampler (Agilent Technologies, Wilmington DE)

- HPLC column Agilent Eclipse Plus C18 (2.1 × 50 mm, 3.5 µm) (Agilent Technologies, Wilmington DE) or comparable
- Agilent TOF MSD model 6200 (Agilent Technologies, Wilmington DE)

8.2 Laboratory Supplies

- Variable volume standard pipettors (10 – 100 µL, 100 – 1000 µL, and 500 – 5000 µL)
- Variable volume repeat pipettors (Eppendorf Repeater Plus)
- RNAase and DNAase free Pipette tips (Genessee Scientific, San Diego, CA)
- Sterile Eppendorf repeat pipettor tips (50 mL)
- Polypropylene autosampler vials and caps (Laboratory Supply Distributors Corp., Mt. Laurel, NJ)
- Ultra-high purity grade compressed nitrogen, (Holox Co., Norcross, GA)
- Solvent bottles
- Permanent markers

8.3 Chemicals and Reagents

- HPLC-grade methanol (MeOH), acetonitrile (ACN) and isopropyl alcohol from Burdick-Jackson (Muskegon, MI) or comparable
- Deionized water from a Barnstead EASYpure UV/UF compact reagent grade water system (Dubuque, IA)
- Ammonium acetate from Sigma-Aldrich Chemical (St Louis, MO)
- Ammonium formate from Sigma-Aldrich Chemical (St Louis, MO)
- Agilent 5 mM purine in acetonitrile:water Agilent part # 18720242
- Agilent 2.5 mM HP0921 in acetonitrile:water Agilent part # 18720241
- Agilent ESI-L Low concentration Tuning mix Agilent part # G1969-85000 (100 mL)

9.0 Procedure

9.1 Cleaning of Labware and Surfaces

9.1.1 Wash all glass equipment used in the procedure using a laboratory-grade dishwasher.

9.1.2 Rinse all glass equipment with methanol immediately before use.

9.1.3 Rinse beakers that will contain aqueous reagents once with deionized water.

9.1.4 Cover all laboratory bench surfaces used in the procedure with clean bench-top laboratory paper.

9.2 Overview of Sample Analysis

Each sample analysis set consists of the following:

- Double blanks (n = dependent on number of samples to be run; at a minimum run before and after each set. In addition interspersed in between unknown samples not to exceed once every 10 unknown samples) mobile phase solvents at ratio equal to starting conditions of gradient.
- Method blank (n≥1) (procedural blank)
- Matrix blanks (n≥1) (if available; matrix blank should match unknown samples being analyzed)
- Calibration curve standards if applicable (n≥6) (analytes of interest spiked into blank matrix if available; otherwise multiple extracted procedural blanks)
- QC samples (n= generally 10% of the number of unknown samples) (either QCs made from of a purchased SRM or spiked in to a blank matrix and run prior to assay to generate target statistics then with sequential analytical batches to assure quality control)
- Unknown samples (typically 1 to 50 samples, each consisting of extracts from environmental or biological samples)
- Replicates (n = generally 10% of the number of unknown samples)

All method and matrix blanks, QCs, calibration standards, replicate and unknown samples are subjected to the same sample preparation and analysis. The following procedure assumes that all method and matrix blanks, QCs, calibration standards, and unknown fish samples have already extracted and cleaned up via the SOPs mentioned in section 2.0.

9.3 Placing Samples in Autosampler Vials

The condensed organic solvent (generally methanol or acetonitrile) eluate is vortexed and placed in a polypropylene autosampler vial containing 2 mM ammonium acetate buffer. The ratio of solvent:buffer should approximate the starting conditions of the mobile phase of the gradient used. Each vial is then capped and vortexed to ensure mixing of the organic sample with the aqueous buffer. The bottom of each vial is checked for air bubbles, which can be removed by tapping on the vial. Samples are then placed in the HPLC autosampler and analyzed by LC/TOFMS.

9.4 HPLC/TOFMS Analysis

Samples were analyzed using an Agilent 1100 HPLC (Agilent Technologies, Palo Alto, CA) interfaced with an Agilent LC/MSD TOF model # G1969A (Agilent Technologies, Palo Alto, CA)). A 45-minute gradient HPLC run (Table 2) was used with a mobile phase components of MeOH and 2 mM formate or acetate buffer at a flow rate of 300 µL/min. The HPLC column was an Agilent Eclipse Plus C18 (2.1 × 50 mm, 3.5 µm) with an injection volume of 40 µL. A comparable HPLC column may be substituted. Electrospray ionization was used in the mass spectrometer source, which was maintained at 325ºC. Depending on

the polarity of the instrument and the mobile phase used, specific reference mass ions are monitored for during analytical runs due to continual infusion for mass correction. See Table 1 for reference ions m/z.

Table 2. The gradient program used on Agilent 1100 HPLC system for the NTA and suspect screening analysis of environmental samples should be kept consistent to allow for chromatographic separation of matrix interferences from analytes of interest and to allow for comparison between sample extracts from different media. However, this gradient may be altered to suit individual samples sets if needed.

Time (min)	Flow Rate (mL/min)	%A[a]	%B[b]
0	0.2	75	25
25	0.2	20	80
40	0.2	0	100
45	0.2	0	100
Post time (4 minutes)	0.2	75	25

[a]Mobile phase component A consisted of 2 mM ammonium format or acetate in deionized water
[b]Mobile phase component B consisted of methanol; acetonitrile may be used if desired for additional assay if needed.

9.4.1 HPLC/TOFMS Analysis

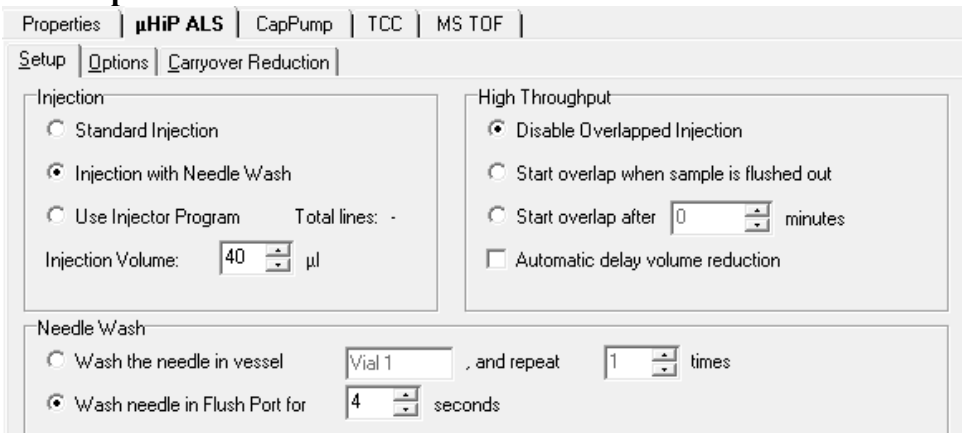
Specific components of the TOFMS Methods are broken up into 4 tabs on the Mass Hunter software. The simplest way to replicate the methods employed are to replicate the setting in the software. The component tabs are:

1) uHiP ALS (the autosampler settings)
2) CapPump (the HPLC pump settings)
3) TCC (the column compartment settings)
4) MS TOF (the TOF mass spectrometer settings)

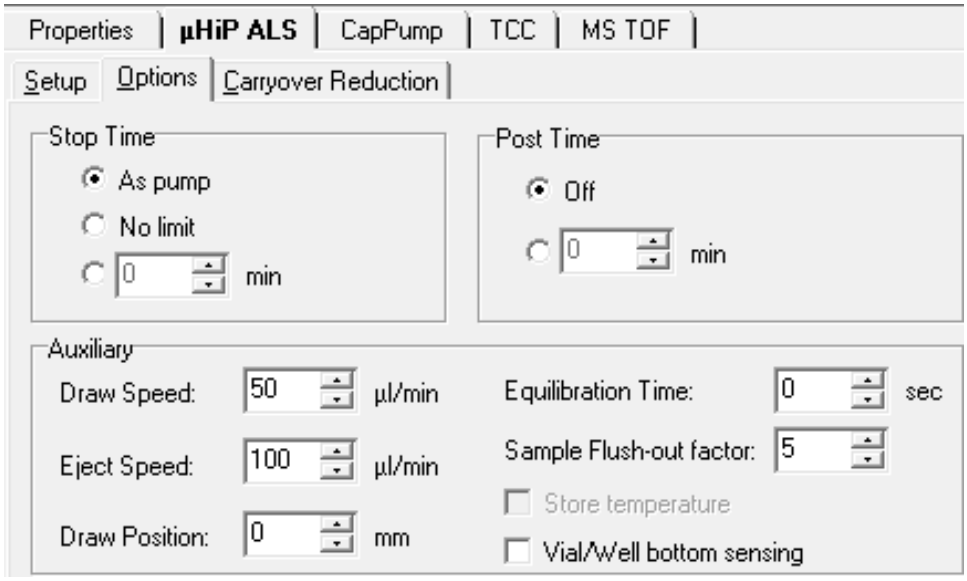
Within each method tab are several tabs for additional settings. This section is organized by component section, then by additional tabs as screen shots.

uHiP ALS Tab

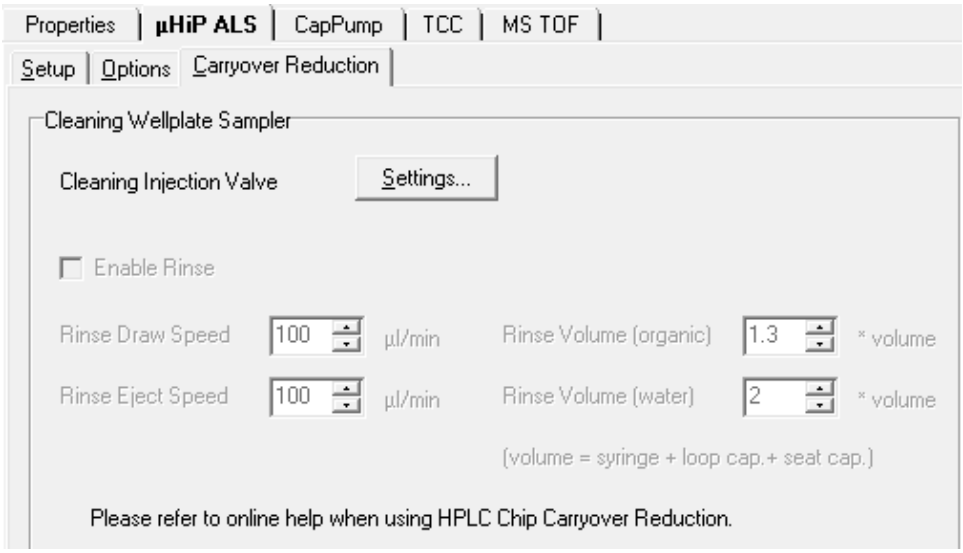
1. Setup Tab



2. Options Tab

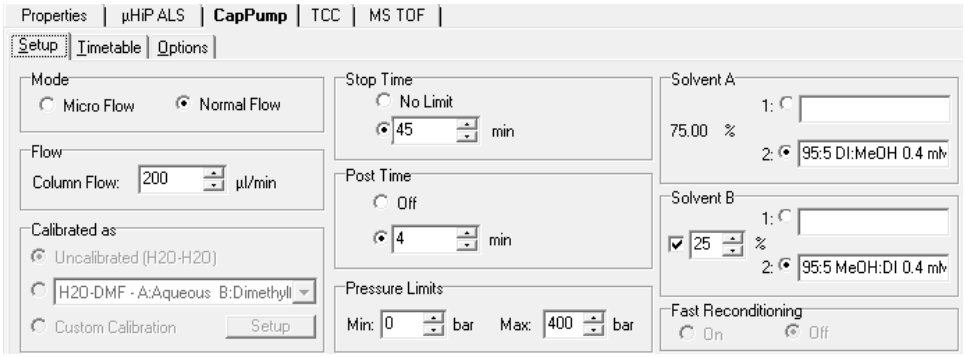


3. Carryover Reduction Tab

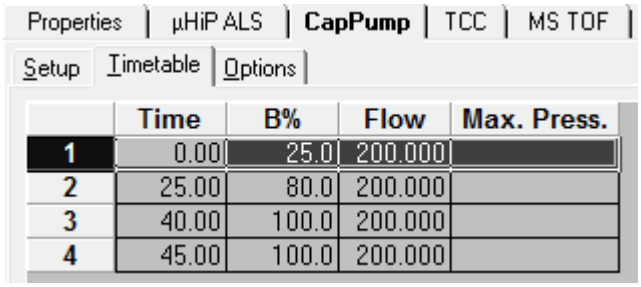


CapPump Tab

1. Setup Tab

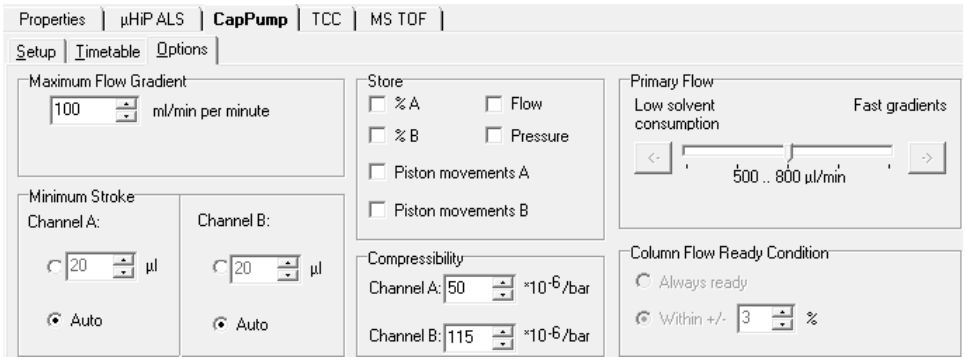


2. Timetable Tab (same as Table 2 information above)



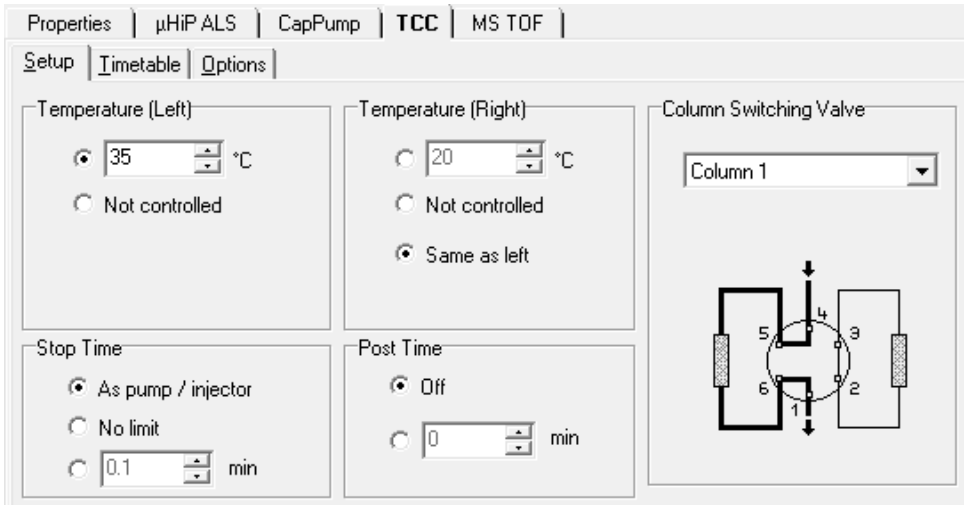
	Time	B%	Flow	Max. Press.
1	0.00	25.0	200.000	
2	25.00	80.0	200.000	
3	40.00	100.0	200.000	
4	45.00	100.0	200.000	

3. Options Tab

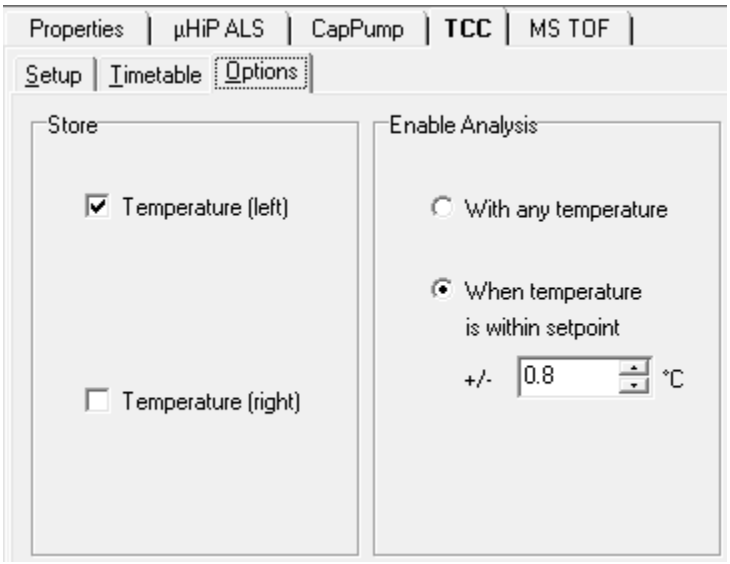


TCC Tab

1.Setup Tab

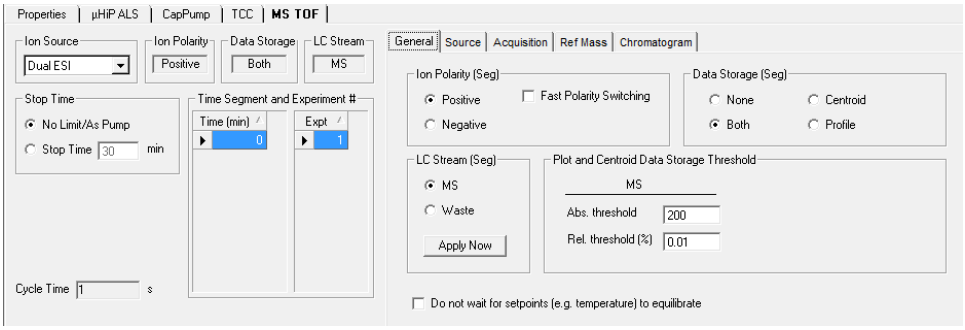


2.Options Tab

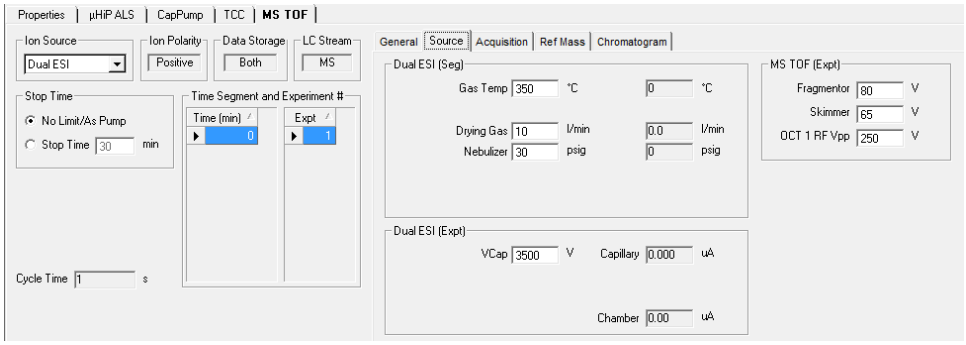


MS TOF Tab

1. General Tab

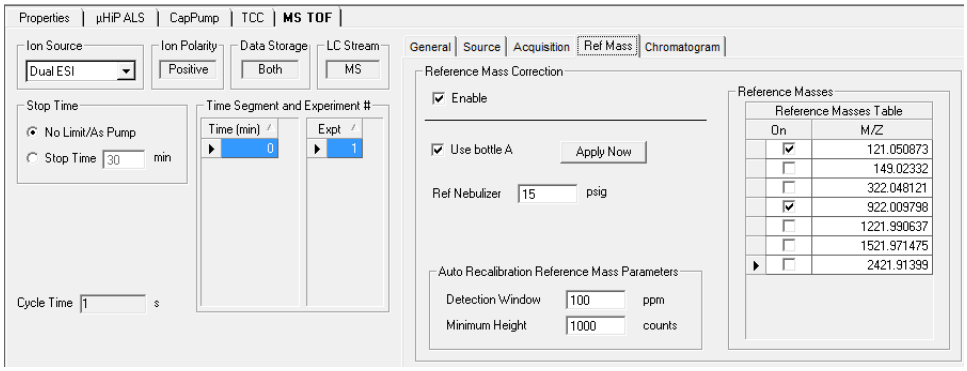


2. Source Tab

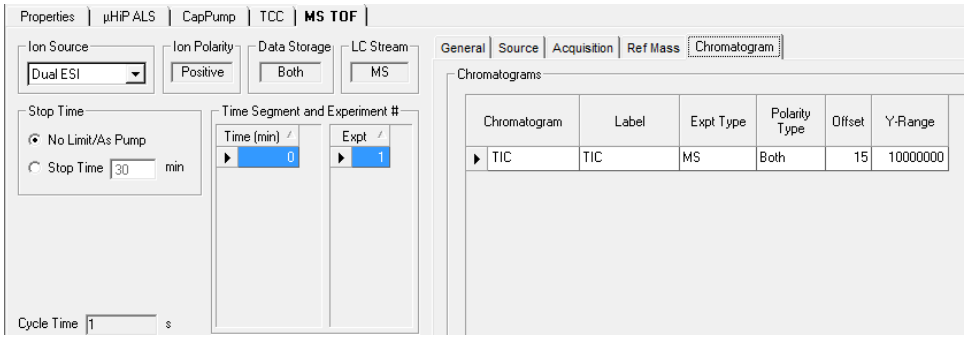


3. Acquisition Tab

4. Ref Mass Tab



5. Chromatogram Tab



9.4.2 Tuning Solutions and Reference solutions for TOFMS Analysis

Critical for any TOFMS or other HRMS analysis is the preparation of proper solutions for calibration of the instrument (tuning solution) and real-time mass correction during any analytical run (reference solutions). The tuning solutions and reference solutions are made up using Agilent stock solutions that can be purchase for an authorized vendor. See section 8.3 for further details.

Tuning Solution

- Agilent ESI-L Low concentration Tuning mix Agilent part # G1969-85000 (100 mL)

Positive Mode Dual ESI (use Undiluted)

Negative Mode Dual ESI (25 mL undiluted + 75 mL of 95:5 Acetonitrile:DI water)

Reference Solution

These solutions are required for real-time mass correction of all generated spectra. These are starting conditions for reference solutions. However, additional solution alteration may be required if the signal is too high or too low depending on the instruments response and mode being used. It is critical that the reference solution m/z be able to be seen by the TOFMS without saturation of the detector. Generally, these values should be between 50,000 and 500,000 counts to be in this range.

- Agilent 5 mM purine in acetonitrile:water Agilent part # 18720242
- Agilent 2.5 mM HP0921 in acetonitrile:water Agilent part # 18720241
- ***additional reference compound added by RTP lab; Not Agilent reference

solution; 1000 ng/uL tetrahydro perfluorononanoic acid (THPNA)****

Positive Mode Dual ESI
500 mL of Acetonitrile:DI water (90:10)
1.5 mL of 5mM purine solution (see above)
750 uL 2.5 mM HP0921solution (see above)

Negative Mode Dual ESI
1000 mL of Acetonitrile:DI water (90:10)
300 uL of 5mM purine solution (see above)
150 uL 2.5 mM HP0921solution (see above)
100 uL of 1000 ng/uL solution of THPFNA

9.5 Batch order and equilibration

Prior to any analytical runs the HPLC/TOF MS is equilibrated for at least 15 minutes with the HPLC system running to waste and the TOF MS system in "On" mode. After equilibration a minimum of 2 double blanks are run prior to any analytical batch to ensure that mobile phase components and the instrument is sufficiently equilibrated. If contamination is determined, appropriate actions are taken to rectify the situation before analysis continues. Once the instrument is determined to be ready for analysis, samples are run in the order of double blanks, method blank, matrix blank, and calibration curve (if needed). After the highest calibration curve point a double blank is run prior to the next sample to ensure carry-over is not an issue. From here on out, QC samples, unknowns and duplicate samples are run in sets of 10 seperated by a double blank until all samples have been run. After a double blank the entire calibration curve is then run again at the end of the analytical run (if needed), followed by multiple double blanks to prepare the system for the next analyst or assay.

9.6 Standard Calibration Curve and Calculations

In general calibration curves are not generally run with NTA and suspect screening analysis. However, at times it is necessary to calibrate for a particular compound of interest, or a series of compounds. This may be used as a semi-quantitative analysis as the analytes of interest generally far exceed in numbers the authentic standards available. In this instance it may be deemed necessary for a general calibration curve of multiple analytes be used for the calibration of analytes found via NTA or suspect screening. A calibration curve of 6-points should be sufficient to get adequate response over the linear response range of the analytes. However, since most analytes do not have authentic standards, conclusions drawn concerning quantitation should be used with caution.

In another application it is possible to use a one point calibration as a point estimate of compounds found via NTA or suspect screening. In this instance an

analyst could use a SRM or a mixed calibration solution with many compounds (100 or greater) to get a point estimate. In the mixed standard approach it is advisable for the standard to be run a two different concentrations (preferably serial dilution: 10:1) and not the response of the analyte being 10x between dilution. Again as before caution should be used in making conclusion about quantitative amounts, and should only be used when estimation of concentrations is needed.

9.7 Nontargeted Screening and Suspect Screening

Generally after a set of samples is run on the HPLC TOFMS system the data processing needs to be accomplished offline through a series of procedures. These procedures are designed to look for features in a sample that match a chemical in a database (suspect screening), or to find chemicals in a sample for further structure elucidation (NTA). A feature is defined as (an accurate mass, a RT and an integrated peak area). The procedures that follow are the best practices to date that have been employed for each approach. However, each step in this procedure is open to further refinement and should be seen as a starting off point for post run analysis.

For NTA a molecular feature extraction must be run. For suspect screening one can skip the MFE step and use the Find by Formula algorithm, or run the suspect screening step (via identify compounds) against the chemicals found via MFE. If the MFE step is not used prior to performing suspect screening, the analysts will only ever find chemicals listed in the databases used. In this application, if that is acceptable suspect screening depends upon the database chosen. A culmination of the chemicals found form the various databases used (DSS-TOX, PCDLs and .CSV files) can be prepared in bulk.

The Generate Formula algorithm can be used to produce candidate formulas for a spectral peak or for compounds found via the MFE. The user may input elements of choice via the “allowed species” tab. Refer to section 9.7.1.4 for screenshots of this procedure. Once all of the parameter for formula generation are selected, a target spectra selecting the first spectral peak of the cluster via left mouse click and drag is chosen and the “play button” is selected to generate a formula for the spectral cluster. The first ion in the cluster must me the monoisotopic mass peak. Generally this is the largest peak. However, in some instances (high chlorinated or brominated compounds) third peak in the cluster is the largest.

9.7.1 Molecular Feature Extraction

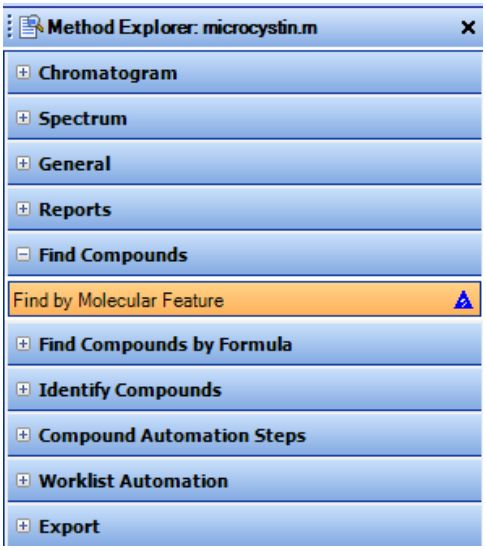
The intention of the MFE algorithm is to identify “features” in the total ion chromatogram (TIC) that require further attention. This MFE process has many variables that may be changed. This procedure is the currently employed set of variables that have been used. However, many of these variables may be changed

to user specific settings for a given project's application. For instance, one could tradeoff speed and detail by altering parameters such as "minimum signal intensity threshold" or "only viewing the top 100 features". It is dependent upon the analyst to determine if the parameters should be altered.

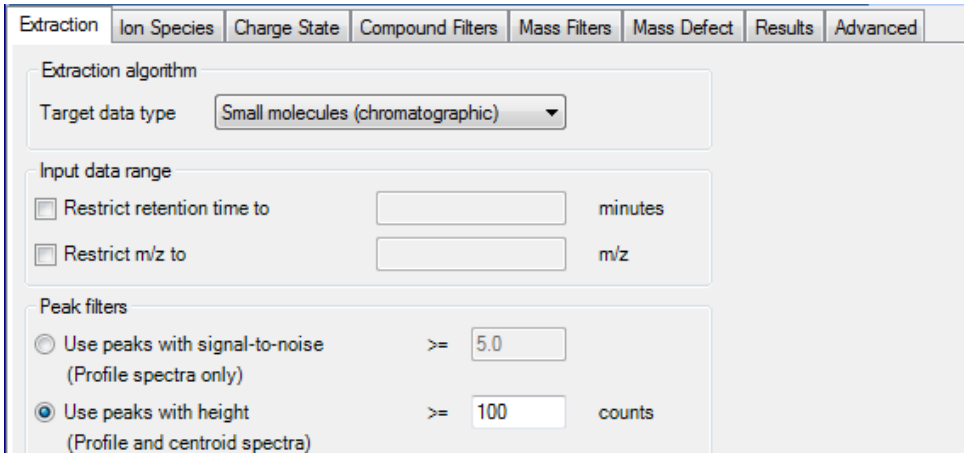
It is highly advisable that the data processing method be saved and dated in a similar fashion as the data files being processed. In this instance, the analyst can go back in the future and observe the parameters that were chosen for the post-hoc analysis.

Example:
Worklist: 4-18-16 Cape Fear water sample
Data Files: 4-18-16 Cape Fear water samples D.01 – D0x.
Data Analysis Method: 4-18-16 Cape Fear water data analysis

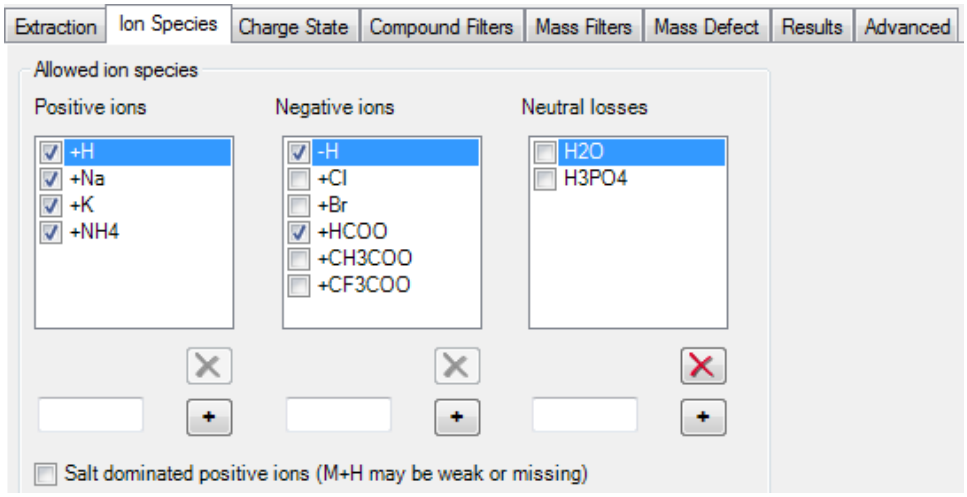
9.7.1.1 Method Explorer: Find Compounds-Find By Molecular Feature



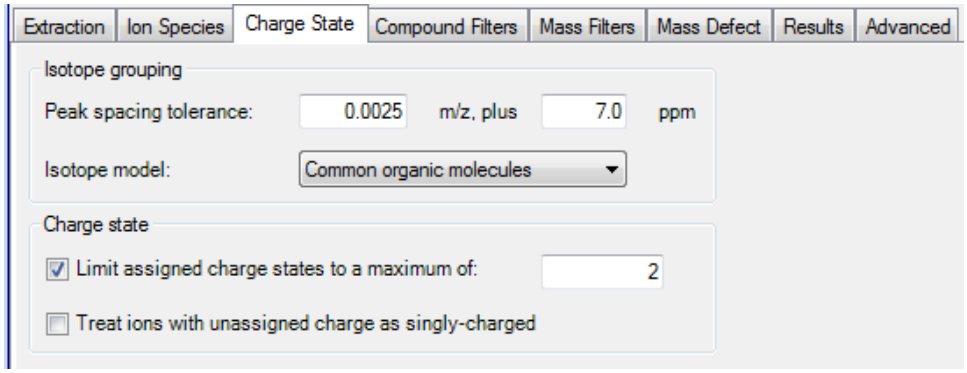
Extraction Tab



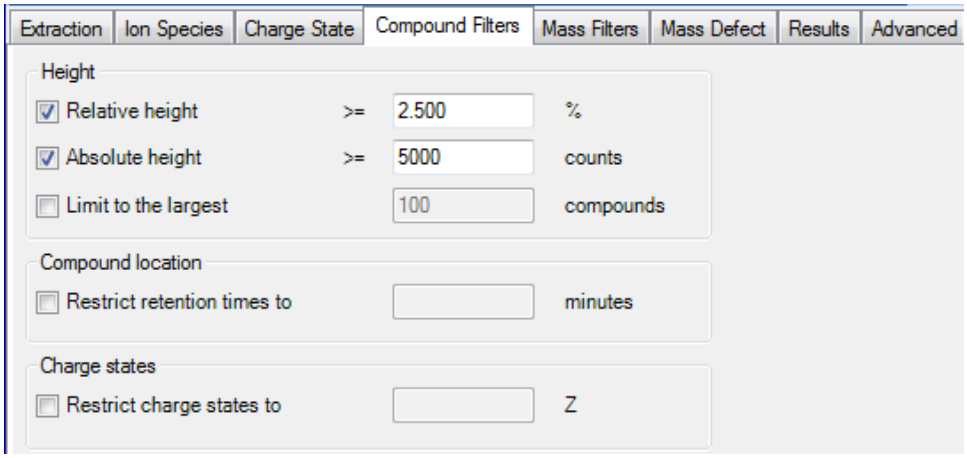
Ion Species Tab



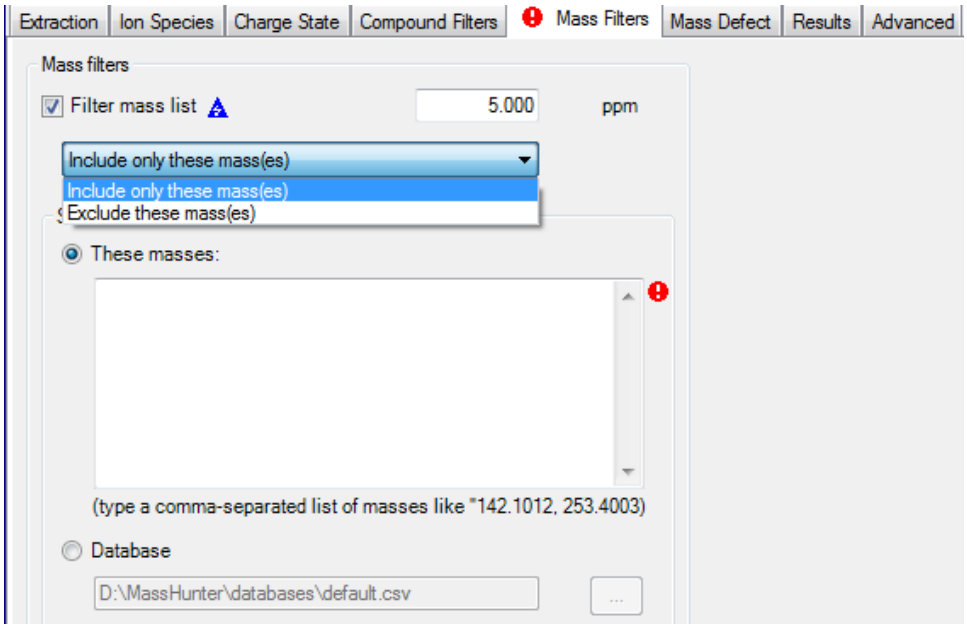
Charge State Tab



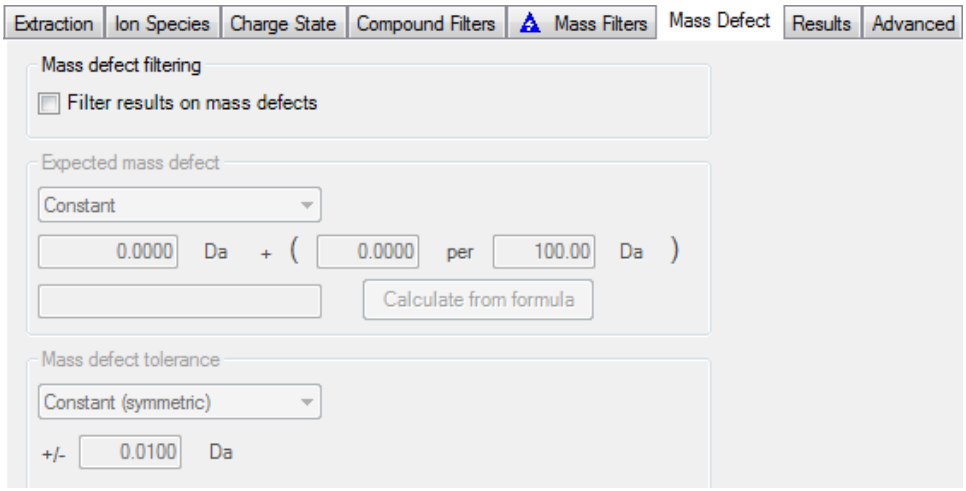
Compound Filters Tab



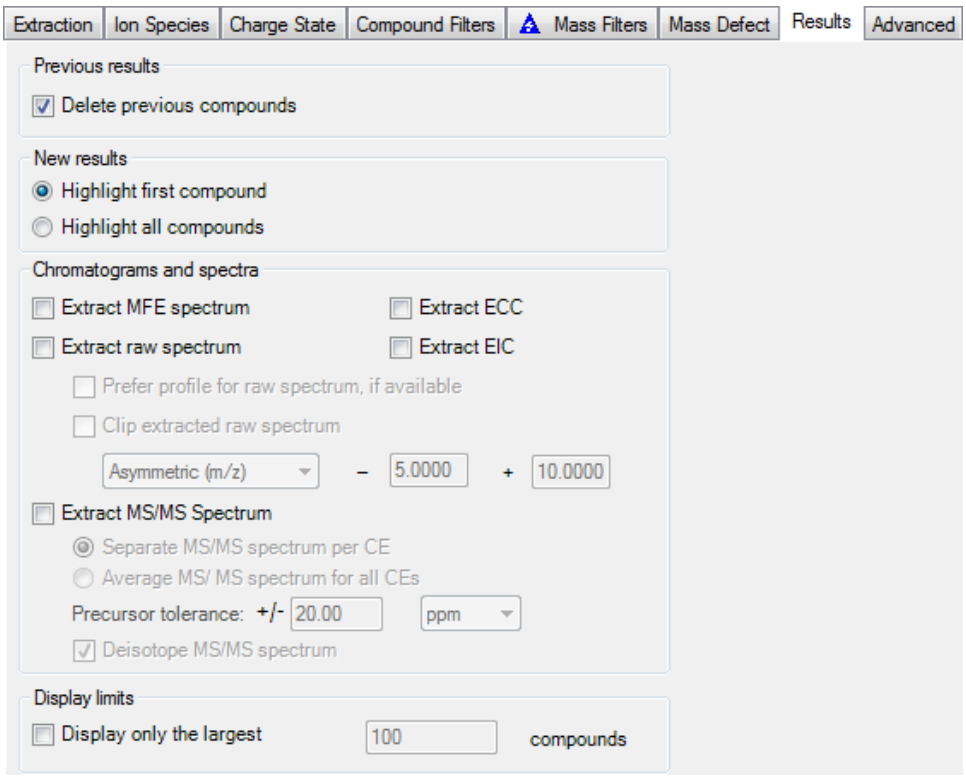
Mass Filters Tab



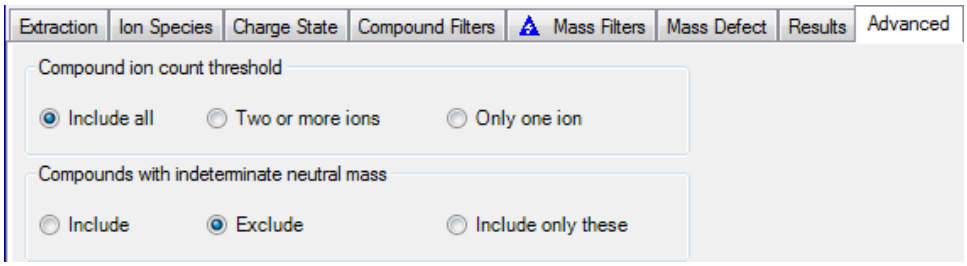
Mass Defect Tab



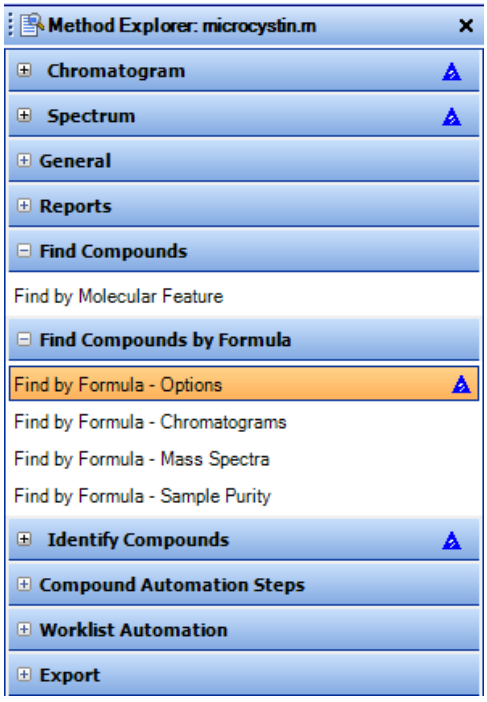
Results Tab



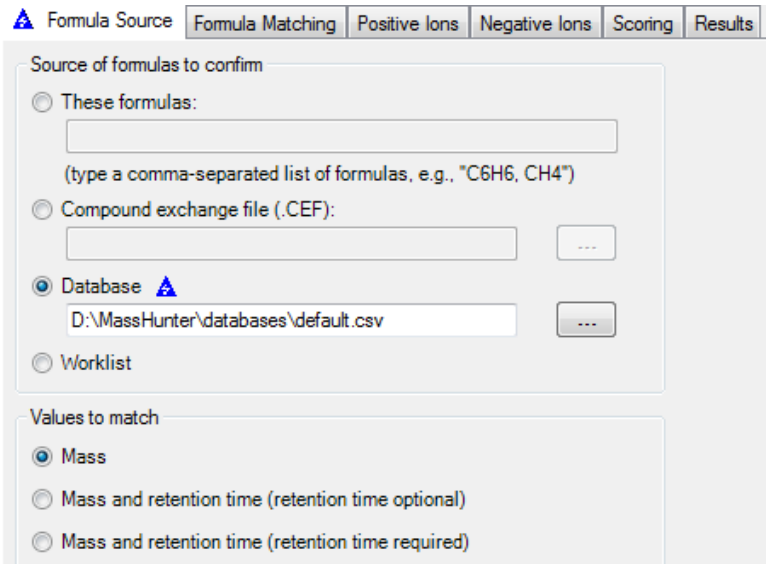
Advanced Tab



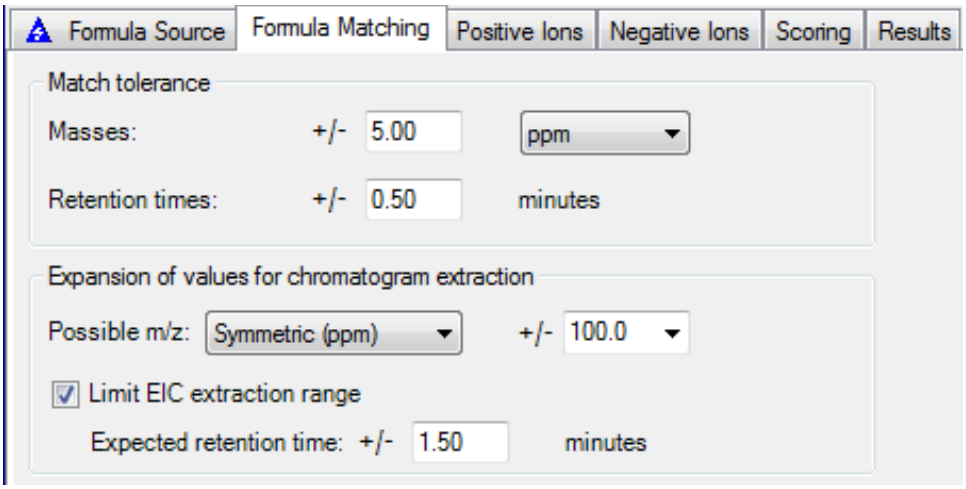
9.7.1.2 Method Explorer: Find Compounds by Formula-Find By Formula Options



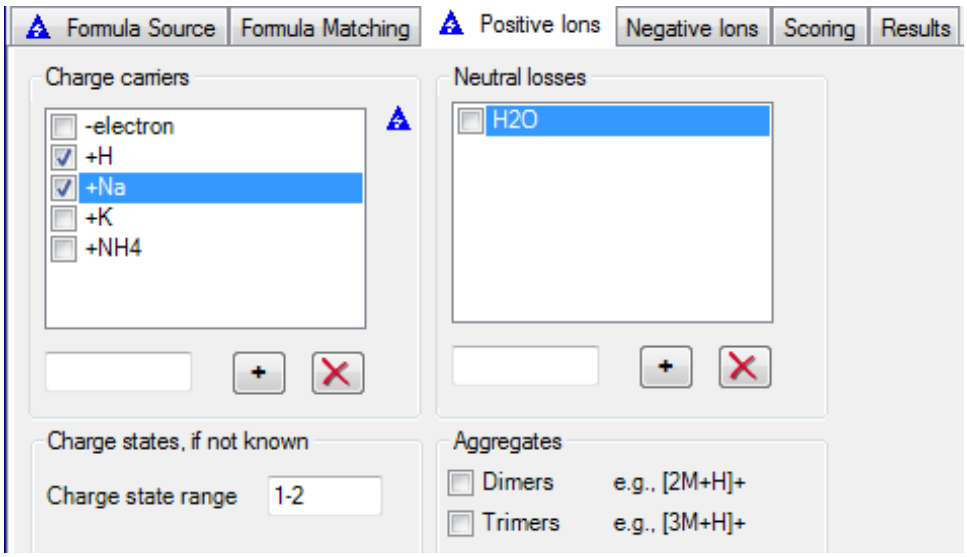
Formula Source Tab



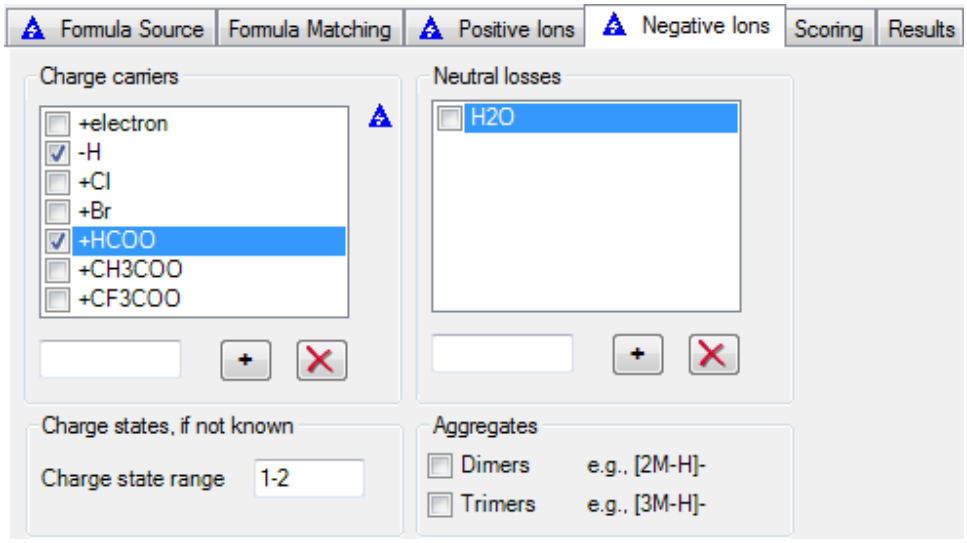
Formula Matching Tab



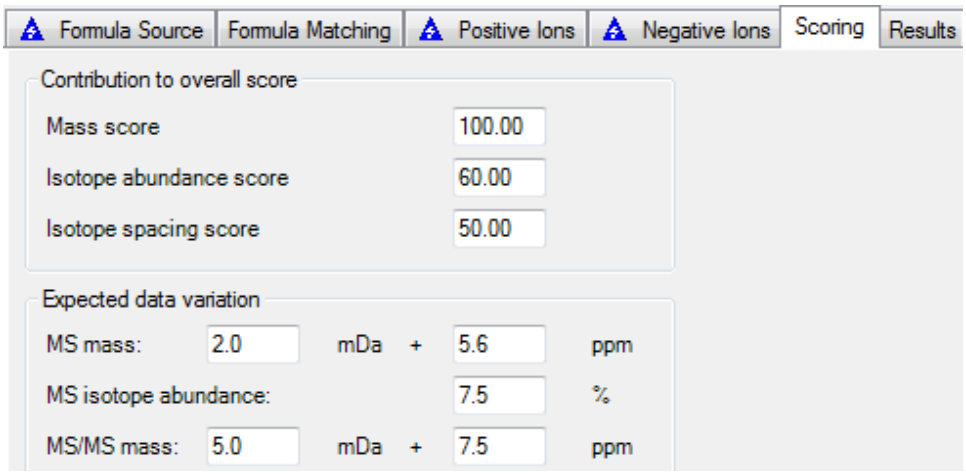
Positive Ions Tab



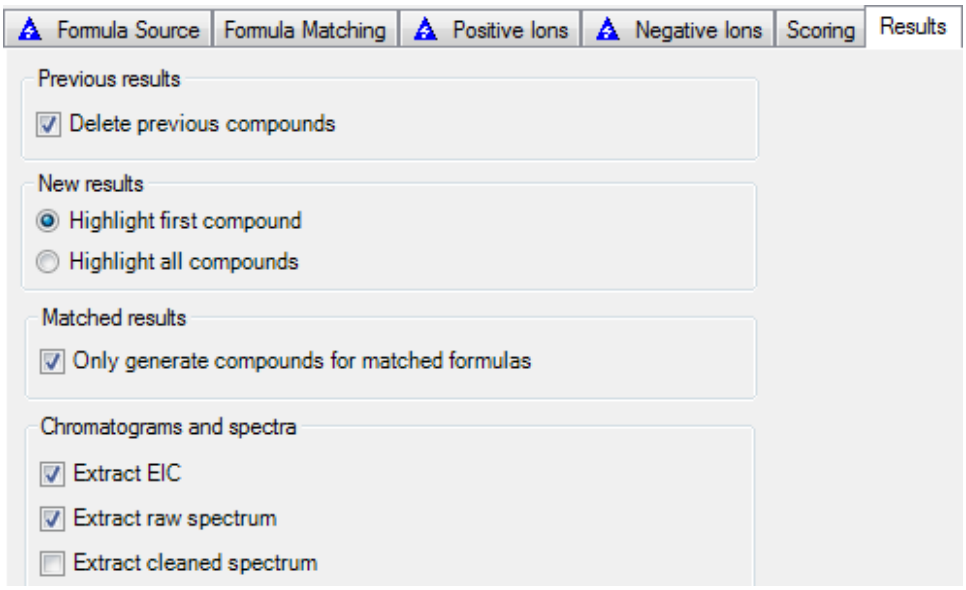
Negative Ions Tab



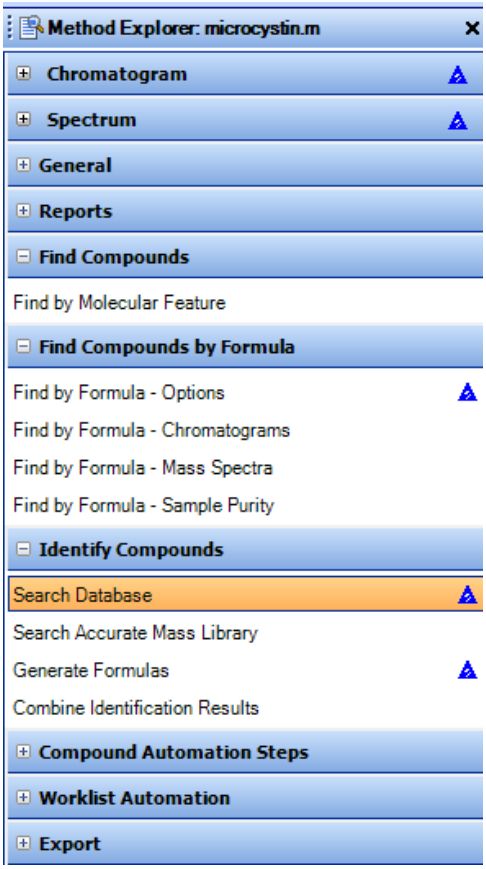
Scoring Tab



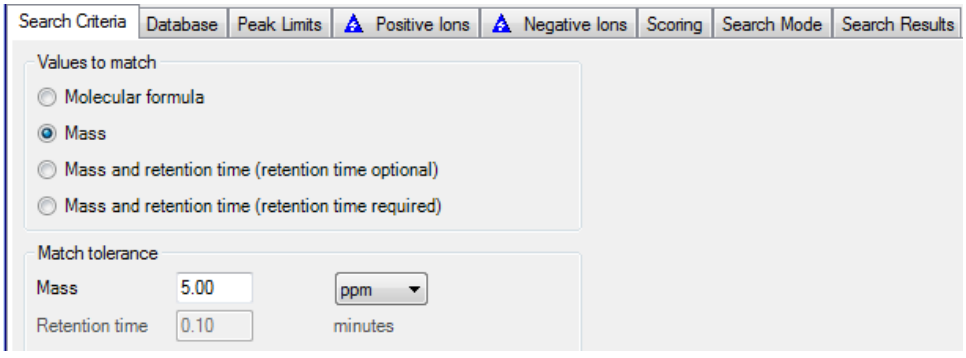
Results Tab



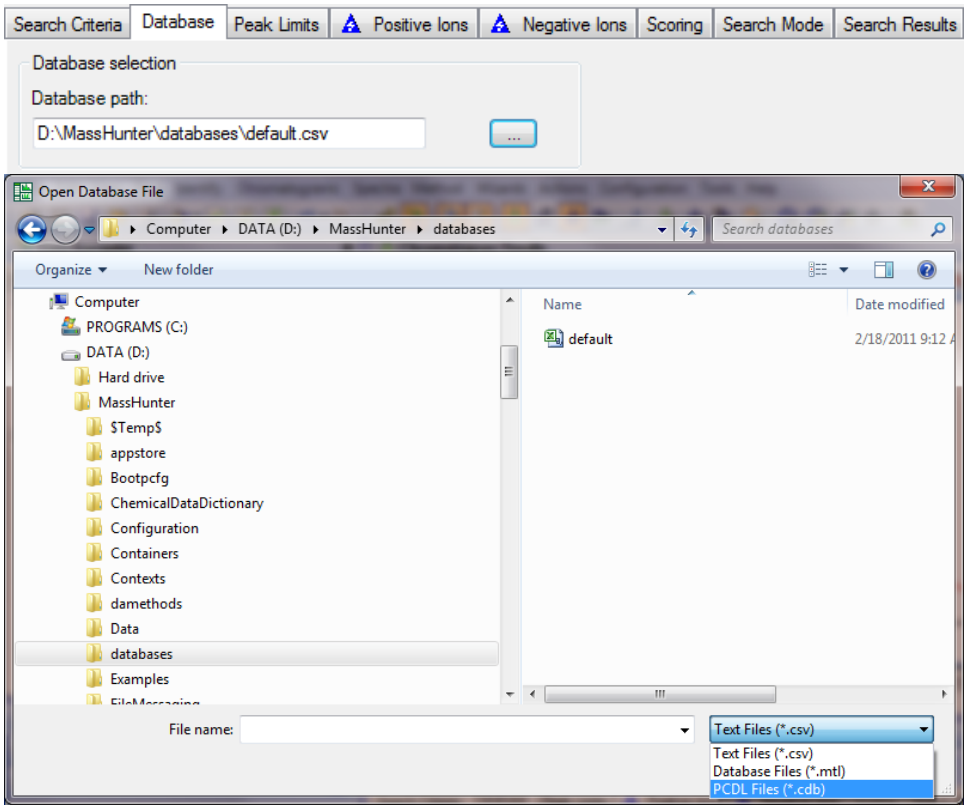
9.7.1.3 Method Explorer: Identify Compounds-Search Database



Search Criteria Tab

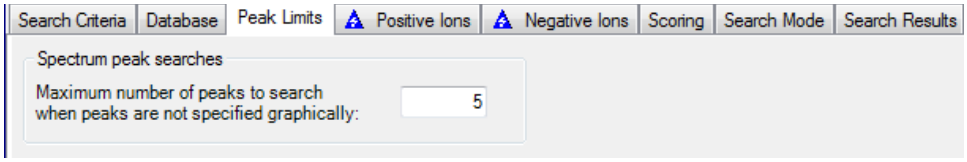


Database Tab

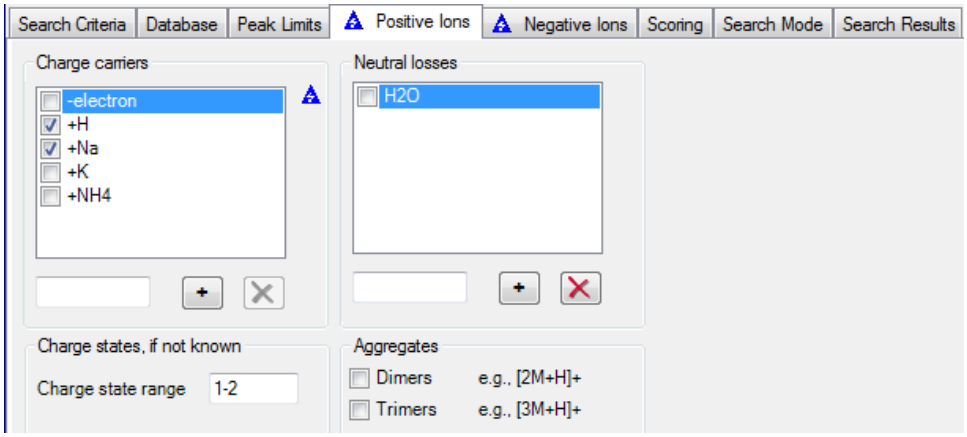


One can choose a .csv, .mtl or a .cdb (PCDL file) here in dropdown menu.

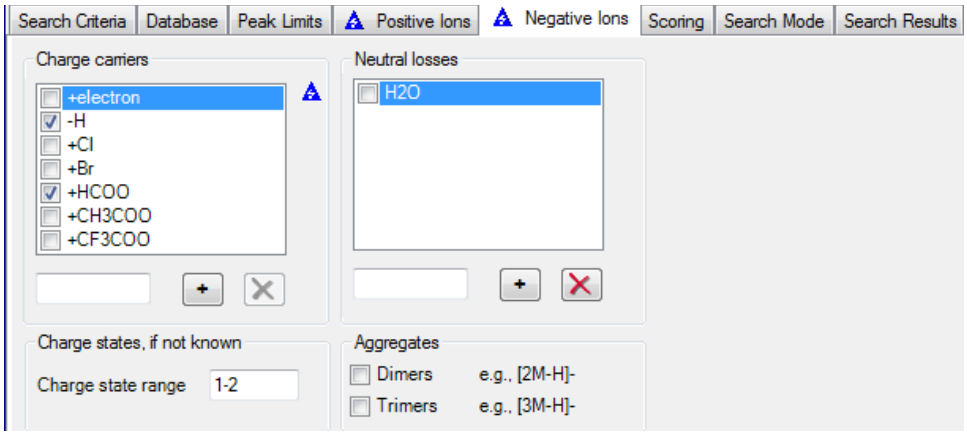
Peak Limits Tab



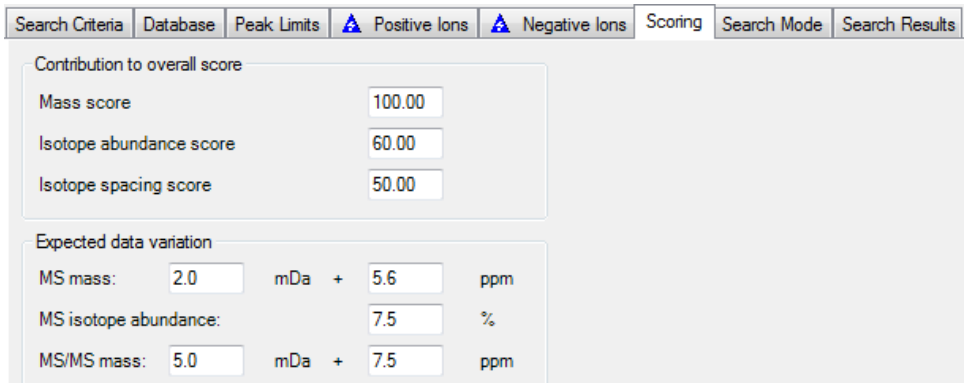
Positive Ions Tab



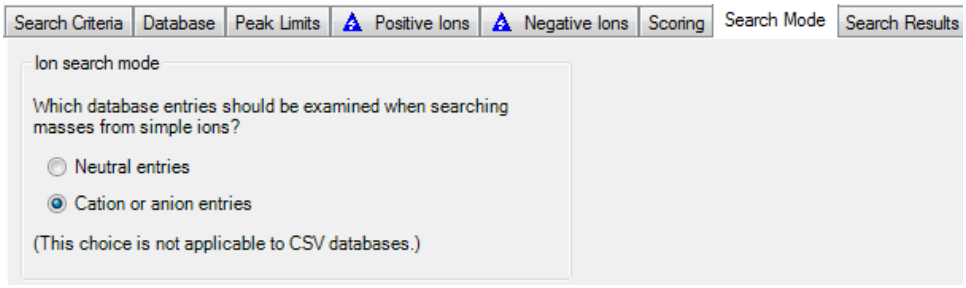
Negative Ions Tab



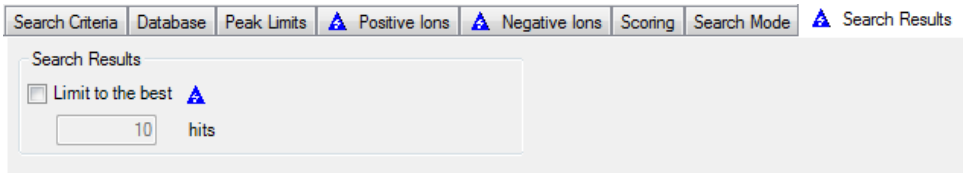
Scoring Tab



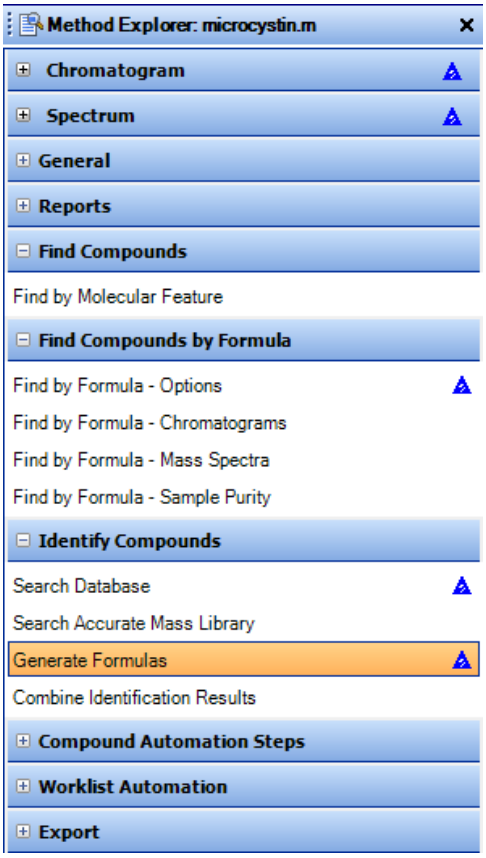
Search Mode Tab



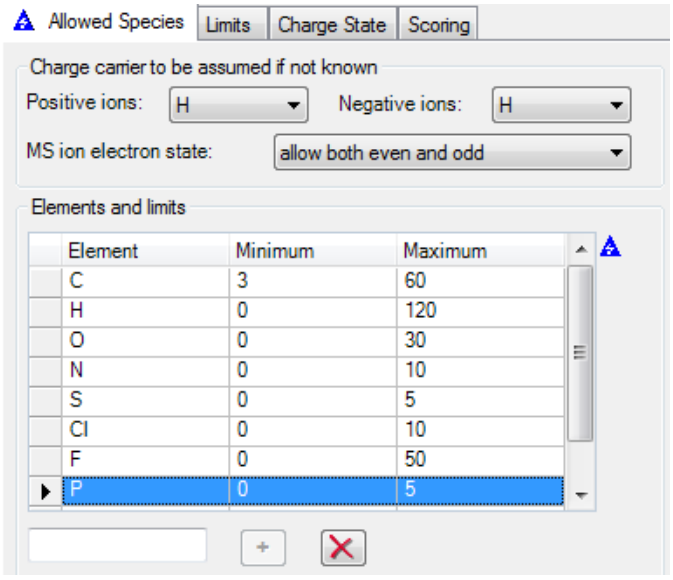
Search Results Tab



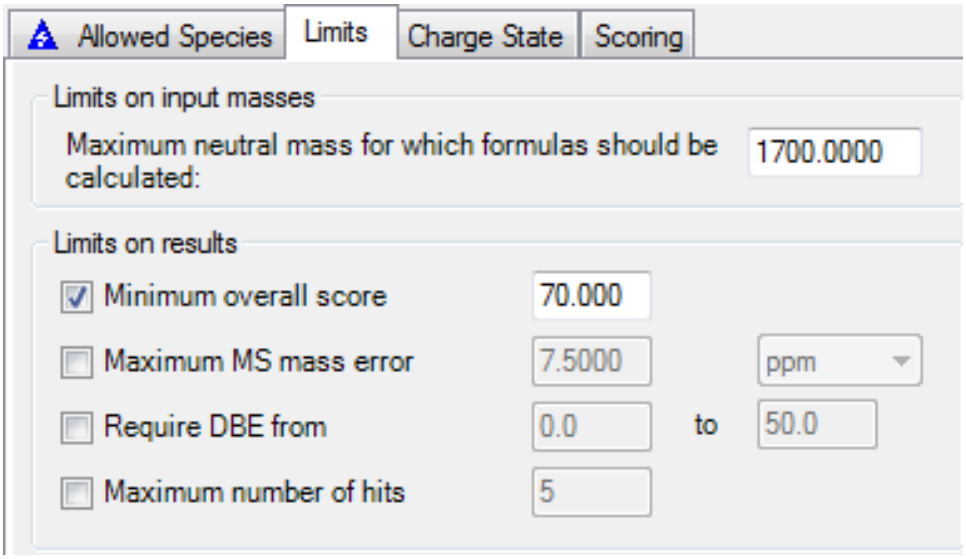
9.7.1.4 Method Explorer: Identify Compounds-Generate Formulas



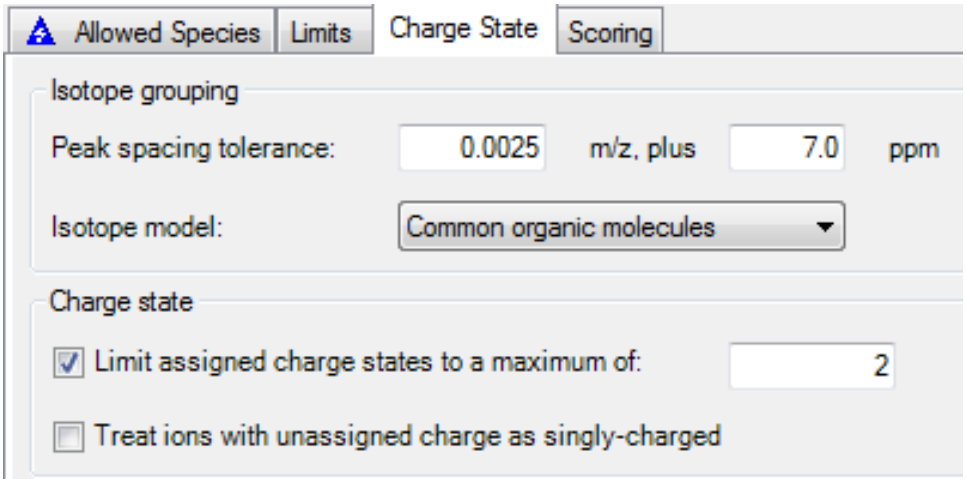
Allowed Species Tab



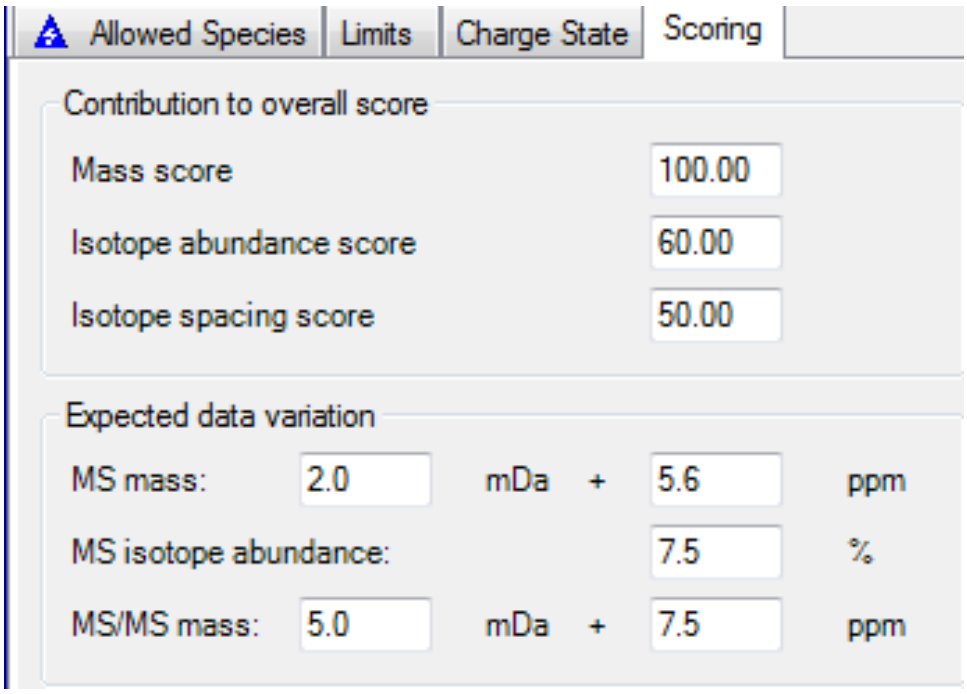
Limits Tab



Charge State Tab



Scoring Tab



9.8 Waste Management

All laboratory waste is disposed of as directed by the EPA-RTP Safety, Health, and Environmental Management Office.

9.8.1 Liquid Waste

Liquid waste generated during sample preparation is collected in a waste beaker during sample preparation and is emptied into a 4L empty solvent bottle that is designated and labeled for waste collection. Staff from Chemical Services are contacted and come to the lab to collect waste containers that are full.

9.8.2 Solid Waste

Solid waste generated during sample preparation is collected in beakers, plastic garbage bags, or burn boxes labeled for non-hazardous waste during sample preparation. All solid waste that is not collected in burn boxes during sample preparation is subsequently emptied into the burn box labeled for non-hazardous waste after sample preparation. Staff from Chemical Services is contacted to remove burn boxes that are full of solid laboratory waste.

10.0 Data and Records Management

10.1 Laboratory Record Book

Records of the preparation of samples, blanks, calibration standards, and QCs will be retained in a laboratory record book (LRB) that is kept by the individual conducting the analysis. This LRB will contain a record of all sample preparation activities and any other data that may be used to interpret results. All samples will be recorded in the LRB by a unique sample ID. The date of extraction and amount of internal standard/extraction solution made on each day of analysis will be recorded in the LRB. Data generated from analysis of samples will also be placed in the LRB. Generated instrumental data are regularly backed up on redundant storage space.

10.2 Location of LRB

The LRB will be retained in the laboratory (or office area) where these operations are performed until the conclusion of the study and will be archived in a secure room for three years after completion of the study according to EPA records policy.

11.0 Quality Control and Quality Assurance

Quality control samples are prepared with each batch of samples. Depending upon the assay being conducted this may be an SRM, spiked blank matrix or a sample that is simply run with every assay. This sample will be used to determine batch to batch consistency. As a measure of the accuracy of the analysis, the percent accuracy of the analytical value, relative to the pre-determined target value are calculated and reported along with the data as an indication of control when available. For NTA and suspect screening assays QA/QC samples are generally used for a different purpose. The purposes may include but are not limited to: batch to batch RT matching, as single point calibration of known compounds in the samples, for future anchoring with datasets not yet run. These QA/QC samples serves a variety of purposes, however it is not in the true sense the same as a QA/QC samples run in traditional analytical assays.

12.0 References

1. EMAB-112.0- Extraction and Analysis of Perfluorinated Compounds (PFCs) from Surface Waters by High Performance Liquid Chromatography (HPLC)-Tandem Mass Spectrometry (MS/MS)
2. MDAB-23.0- Standard Operating Procedure (SOP) For Extraction And High Performance Liquid Chromatography/Mass Spectrometry Analysis Of Perfluorinated Acids And Sulfonates From House Dust

3. MDAB-077.0 - Extraction of Perfluorinated Compounds from Fish Liver Homogenate
4. PHCB-033-SOP-01 - Extraction and Analysis of SVOCs in Tire Crumb Rubber Samples
5. Agilent 6200 Series TOF LC/MS Techniques and Operation Course Number R1874A Volumes, I, II and III. Qual B.04.00 SP1; Quant B.04.00 SP3. Student Manual. July, 2011

Note: For copies of SOPs, please call the PI, Dr. Mark J Strynar (919-541-3706) or email at Strynar.mark@epa.gov